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| 14. ABSTRACT The most significant challenge in managing localized prostate cancer is the decision of whether or not it needs to be treated. Nearly 1/2 of prostate cancers diagnosed in the U.S. fall into the low or very low risk category and have little likelihood of causing death. However, it is well known that a significant fraction of low risk cases are misclassified and actually have occult high-risk features or are destined to progress to high-risk disease. Therefore a critical need in localized prostate cancer is the development of biomarkers that predict occult or incipient aggressive disease in the low-risk population. | | | | | |
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Validation of Biomarkers for Prostate Cancer Prognosis

Progress Report

Synergy Award: W81XWH-11-1-0381

Co-PIs: Ziding Feng & James D. Brooks

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Introduction

The most significant challenge in managing localized prostate cancer is the decision of whether or not it needs to be treated. Nearly $\frac{1}{2}$ of prostate cancers diagnosed in the U.S. fall into the low or very low risk category and have little likelihood of causing death. However, it is well known that a significant fraction of low risk cases are misclassified and actually have occult high-risk features or are destined to progress to high-risk disease. Therefore a critical need in localized prostate cancer is the development of biomarkers that predict occult or incipient aggressive disease in the low-risk population.

To address this challenge, we formed the multi-institutional Canary Tissue Microarray Project. We have used rigorous clinical trial case/cohort design, taking care to correct for institutional and spectrum biases. Funding from the Department of Defense allowed us to complete construction of the TMAs as well as the necessary infrastructure and begin testing biomarker candidates. With this infrastructure in place, we now have a robust validation platform for testing prostate cancer biomarkers. Based on our success, this resource will be a source for future biomarker validation studies even after the DOD funding has ceased.

The DOD has catalyzed the formation of the infrastructure to support this project and we have now completed or are near completion of several biomarkers. Staining has been completed, slides have now been analyzed and statistical analyses are underway. I requested and received an extension of my half of the award because of my move from Seattle to MD Anderson Cancer Center in Houston. Actually, this will be very beneficial for this project since the next phase for several of the biomarkers is the statistical analysis of the data. My team is actively working on data analyses and communicating back and forth with lab and clinical collaborators and we expect over the next year will complete several projects and should lead to several publications. This will serve as critical preliminary data for us to continue this resource and apply for competitive funding.

Specific Aim 1) To test markers of prognosis on prostate cancer tissue microarrays with associated clinical data.

1.A. Develop work-flow for TMA sharing, image scanning, TMA staining data analysis.

The multi-institutional TMAs have been constructed at all sites. The final TMA cohort is 1326 patients with only 31 patients excluded due to data error. We are in the process of updating follow-up on the TMAs since several years of additional follow-up have been accumulated since the cases were first selected. Patients have been selected at random from the pool of patients who had undergone radical prostatectomy at each of the sites, with special attention to selecting patients with features typical of low-intermediate risk patients seen in contemporary urologic practices. Details of patient selection, statistical considerations, and TMA construction are summarized in our publication in *Advances in Anatomic Pathology* published earlier this year and appended to last year's report. In addition to this cohort, a separate TMA has been constructed from 220 patients who underwent radical prostatectomy at a sister site who have very long term follow-up (up to 25 years) and hard endpoints including metastases and prostate cancer specific death.

Since many of these patients were diagnosed in the pre-and early PSA eras, they are held separately as a validation cohort.

We have completed several stated aims in the proposal with regard to development of work-flow for array sharing, analysis and archiving while some aspects continue to be developed:

1) The Data Transfer Agreement (DTA) was completed between FHCRC and MDACC so the study data could be freely shared and communicated between FHCRC and MDACC. MDACC has established new database to warehouse the study data, receiving and archiving assay data from different labs/groups submitted to this project.

2) We have concluded that TACOMA algorithm as it currently stands, it inadequate for automatic imaging reading. The main reason is that it still requires pathologists to sketch the boundary for cancer cell region. Though Dr. Tim Randolph will continue collaborating with Dr. Richard Levenson to add that functionality by another new software, it wouldn't be available in the life length of this project period to reduce pathologist reading time.

3) Data management and data analysis: We have performed data analyses for all biomarkers whose data has been submitted to MDACC. The details of the findings are summarized below.

1.B. Test candidate biomarkers of prognosis for prediction of recurrence after radical prostatectomy

In our ongoing monthly conference calls, the TMA investigators review progress and review applications for utilizing the TMAP resource. Most applications for use of the TMAs come from within the group, although it is available to the prostate cancer research community broadly and can be accessed by application through the Canary Foundation website (<http://www.canaryfoundation.org>). We have focused on biomarkers that have well characterized, highly performing reagents (e.g. immunohistochemical grade antibodies) and sufficient preliminary data that they could supply prognostic information independent of grade, stage and PSA. We have now completed staining for many of the biomarkers listed in our proposal and are expanding to novel biomarkers discovered since our application.

The primary objective is to correlate these two biomarkers with survival endpoints. Three survival endpoints were of interest: recurrence-free survival (RFS, where event was defined as any recurrence or metastasis or prostate cancer death), disease-specific survival (DSS, where event was defined as metastasis or prostate cancer death), and overall survival (OS, where event was defined as death of any cause).

We will first give an overall summary of the proposed assays and their status, followed by details of the findings not reported in previous progress report.

Summary of Proposed Assays and their Status

| Applicant | Proposed Assay | Status |
|--------------------|------------------------------|--|
| Squire/Troyer | PTEN FISH | Published |
| McKenney/Brooks | ERG | Published |
| McKenney/Brooks | SPINK1 | Published |
| Lotan | PTEN-IF and PTEN-IHC | published and second manuscript submitted |
| Tretiakova | Ki67 | Submitted |
| Brooks | AZGP1 in situ | Submitted |
| Brooks | AZGP1 antibody | Submitted |
| Brooks | MUC1 | manuscript in prep |
| Ayalo/McKenney | stromal quantification (H&E) | manuscript in prep |
| Brooks | ARG2 and CD38 | analysis underway |
| Chatterjee | SULT2B | analysis of subset complete; additional proposal anticipated |
| Drake | N-glycan via MALDI | analysis of subset complete; additional proposal received |
| McKenney | Masson's trichrome | scoring underway |
| True | CD10 | scoring underway |
| McKenney | p63 | scoring underway |
| Brooks/Vakar-Lopez | p27 | manuscript in prep |
| Brooks/Beck | HE4: prognostic model | on hold |
| Meng | MCM2 | reviewed; conditional approval |
| Rohit Mehra | Schlap1 | reviewed; conditional approval |
| Drake | N-glycan via MALDI and other | reviewed; not approved but revision requested |
| Liu | SMAD7 | canceled; slides returned |
| McKenney | Histology pattern | Manuscript in prep |

Updates on completed biomarkers not reported in previous progress reports:

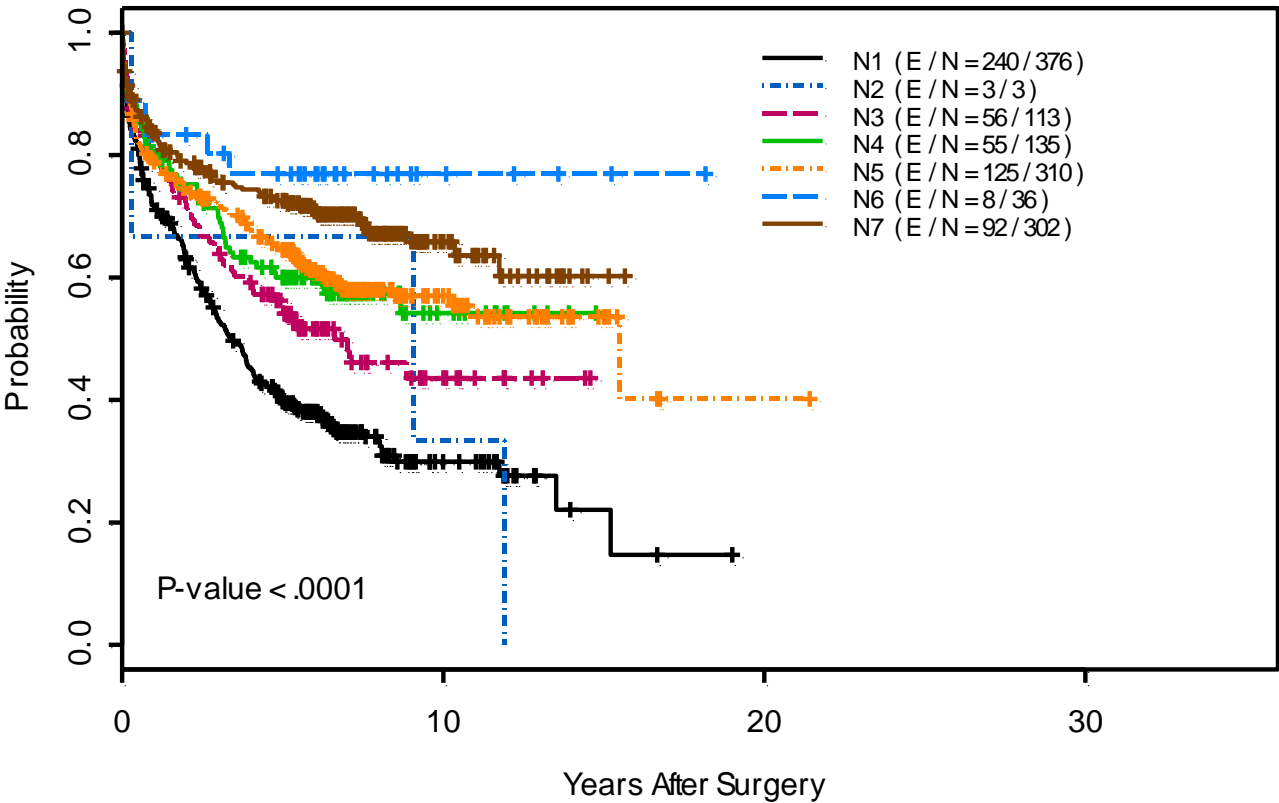
1. Histological pattern is strongly associated with recurrence-free survival (RFS). Manuscript in preparation.

Table 1. Summary of multivariate Cox proportional hazard model for recurrence-free survival (RFS), where an event is defined as any recurrence, metastasis, or prostate cancer death after surgery. The first model used individual histological patterns. The second model used groups of histological patterns. Hazard ratio higher than 1 means better prognosis. Conclusions:

1. In model 1, the presence of Aw and By were significantly associated with better RFS, whereas the presence of S3 was significantly associated with worse RFS. Their effects were adjusted for margin, SVI, ECE, clinical reported Gleason score, and pre-op PSA.
2. In model 2, the presence of Aw or By was significantly associated with better RFS, whereas the presence of Ay1 or Ay2 was significantly associated with worse RFS.
3. Positive margin, SVI, ECE, Gleason higher than 3+4, and higher baseline PSA were significantly associated with worse RFS.
4. The interaction between histological patterns or groups of patterns and clinical Gleason score were not significant (results not shown), meaning that the effect of histological patterns on RFS was the same across all Gleason score groups.

| Endpoint | Factor | Comparison | Hazard Ratio | 95% LCL | 95% UCL | P-value |
|-----------------------------|--------------------|--------------------------------|--------------|---------|---------|---------|
| RFS (N = 939 E = 429) | HistAw | Present vs. Absent | 0.69 | 0.56 | 0.86 | 0.001 |
| | HistBy | Present vs. Absent | 0.78 | 0.62 | 0.98 | 0.03 |
| | HistS3 | Present vs. Absent | 1.56 | 1.08 | 2.24 | 0.02 |
| | Margin | Positive vs. Negative | 1.65 | 1.34 | 2.02 | <.0001 |
| | SVI | Yes vs. No | 2.03 | 1.48 | 2.78 | <.0001 |
| | ECE | Yes vs. No | 1.27 | 1.03 | 1.56 | 0.03 |
| | Gleason (clinical) | 3+4 vs. 6 | 1.23 | 0.97 | 1.55 | 0.08 |
| | | 4+3 vs. 6 | 1.85 | 1.39 | 2.46 | <.0001 |
| | | 8-10 vs. 6 | 1.44 | 1.05 | 1.99 | 0.02 |
| | log(pre-op PSA) | 1 unit increase | 1.41 | 1.21 | 1.65 | <.0001 |
| | | | | | | |
| | Aw/By | Either present vs. Both absent | 0.66 | 0.54 | 0.81 | <.0001 |
| | Ay1/Ay2 | Either present vs. Both absent | 1.52 | 1.08 | 2.15 | 0.02 |
| | Margin | Positive vs. Negative | 1.65 | 1.35 | 2.03 | <.0001 |
| | SVI | Yes vs. No | 2.00 | 1.46 | 2.74 | <.0001 |
| | ECE | Yes vs. No | 1.28 | 1.04 | 1.58 | 0.02 |
| | Gleason (clinical) | 3+4 vs. 6 | 1.23 | 0.98 | 1.55 | 0.08 |
| | | 4+3 vs. 6 | 1.88 | 1.41 | 2.50 | <.0001 |
| | | 8-10 vs. 6 | 1.44 | 1.05 | 1.98 | 0.03 |
| | log(pre-op PSA) | 1 unit increase | 1.41 | 1.21 | 1.64 | <.0001 |

RFS by Histology Pattern



| | | | | | | |
|------------|-----|-----|-----|-----|----|------|
| Year: | 0 | 2.5 | 5 | 7.5 | 10 | 12.5 |
| N.Risk.N1: | 376 | 206 | 131 | 48 | 20 | 7 |
| N.Risk.N2: | 3 | 2 | 2 | 2 | 1 | 0 |
| N.Risk.N3: | 113 | 72 | 53 | 21 | 13 | 4 |
| N.Risk.N4: | 135 | 92 | 68 | 26 | 12 | 4 |
| N.Risk.N5: | 310 | 214 | 180 | 77 | 38 | 21 |
| N.Risk.N6: | 36 | 27 | 22 | 10 | 5 | 3 |
| N.Risk.N7: | 302 | 216 | 192 | 87 | 33 | 14 |

N1: Ex, Ey, Ez, Dy, Dz, Cy, Cz, Bz, Ay1, Ay2 any present
N2: Not N1 and Ew present
N3: Not N1-N2 and Dx present
N4: Not N1-N3 and Bx or Cx present
N5: Not N1-N4 and Az present
N6: Not N1-N5 and Bw or Cw or Dw present
N7: Not N1-N6

2. Positive MUC1 was significantly associated with worse OS after adjusting for Gleason and age. Manuscript in preparation.

Table 2. Summary of multivariate Cox proportional hazard model for recurrence-free survival (RFS, where an event is defined as any recurrence, metastasis, or prostate cancer death), overall survival (OS, where an event is defined as death due to any cause), and disease-specific survival (DSS, where an event is defined as metastasis or prostate cancer death) for Mucin1. Hazard ratio higher than 1 means worse prognosis. Pairwise p-values were provided only if the overall p-value was significant. Conclusions:

1. Positive MUC1 was significantly associated with worse OS after being adjusted for Gleason and age.

| Endpoint | Model | Factor | Comparison | Hazard Ratio | 95% LCL | 95% UCL | Pairwise P-value | Overall P-value |
|----------|-------|-----------------|-----------------------|--------------|---------|---------|------------------|-----------------|
| RFS | 1 | MUC1 | Positive vs. Negative | 1.14 | 0.92 | 1.42 | 0.23 | 0.0001 |
| | | Margin | Positive vs. Negative | 1.64 | 1.32 | 2.03 | <.0001 | |
| | | SVI | Positive vs. Negative | 2.10 | 1.52 | 2.90 | <.0001 | |
| | | ECE | Positive vs. Negative | 1.30 | 1.04 | 1.62 | 0.02 | |
| | | Gleason | 3+4 vs. 6 | 1.20 | 0.94 | 1.53 | 0.14 | |
| | | | 4+3 vs. 6 | 1.92 | 1.43 | 2.58 | <.0001 | |
| | | | 8-10 vs. 6 | 1.50 | 1.07 | 2.09 | 0.02 | |
| | | Log(pre-op PSA) | 1 unit increase | 1.42 | 1.22 | 1.67 | <.0001 | |
| | | | | | | | | |
| | 2 | MUC1 | Strong vs. Negative | 1.39 | 0.96 | 2.01 | N/A | 0.36 |
| | | | Moderate vs. Negative | 1.03 | 0.72 | 1.46 | N/A | |
| | | | Weak vs. Negative | 1.11 | 0.82 | 1.50 | N/A | |
| | | Margin | Positive vs. Negative | 1.65 | 1.33 | 2.04 | <.0001 | |
| | | SVI | Positive vs. Negative | 2.04 | 1.47 | 2.83 | <.0001 | |
| | | ECE | Positive vs. Negative | 1.30 | 1.04 | 1.62 | 0.02 | |
| | | Gleason | 3+4 vs. 6 | 1.20 | 0.94 | 1.52 | 0.15 | 0.0002 |
| | | | 4+3 vs. 6 | 1.91 | 1.42 | 2.57 | <.0001 | |
| | | | 8-10 vs. 6 | 1.48 | 1.06 | 2.06 | 0.02 | |
| | | Log(pre-op PSA) | 1 unit increase | 1.42 | 1.22 | 1.67 | <.0001 | |
| | | | | | | | | |
| | | | | | | | | |
| OS | 3 | MUC1 | Positive vs. Negative | 1.82 | 1.06 | 3.11 | 0.03 | 0.0005 |
| | | Gleason | 3+4 vs. 6 | 0.89 | 0.45 | 1.77 | 0.74 | |
| | | | 4+3 vs. 6 | 1.17 | 0.47 | 2.95 | 0.73 | |
| | | | 8-10 vs. 6 | 3.46 | 1.76 | 6.78 | 0.0003 | |
| | | Age | 1 year increase | 1.07 | 1.02 | 1.11 | 0.003 | |
| | | | | | | | | |
| | 4 | MUC1 | Strong vs. Negative | 1.57 | 0.65 | 3.81 | N/A | 0.11 |
| | | | Moderate vs. Negative | 2.36 | 1.16 | 4.83 | N/A | |
| | | | Weak vs. Negative | 1.52 | 0.67 | 3.44 | N/A | |
| | | Gleason | 3+4 vs. 6 | 0.89 | 0.45 | 1.78 | 0.75 | 0.0004 |
| | | | 4+3 vs. 6 | 1.16 | 0.46 | 2.92 | 0.75 | |
| | | | 8-10 vs. 6 | 3.59 | 1.82 | 7.09 | 0.0002 | |
| | | Age | 1 year increase | 1.07 | 1.02 | 1.11 | 0.003 | |
| | | | | | | | | |
| | | | | | | | | |
| DSS | 5 | MUC1 | Positive vs. Negative | 0.81 | 0.42 | 1.56 | 0.53 | |

| | | | | | | | | |
|-----|---|-----------------|-----------------------|------|------|-------|--------|--------|
| | | Gleason | 3+4 vs. 6 | 2.70 | 1.18 | 6.18 | 0.02 | 0.001 |
| | | | 4+3 vs. 6 | 3.68 | 1.39 | 9.78 | 0.01 | |
| | | | 8-10 vs. 6 | 6.54 | 2.59 | 16.49 | <.0001 | |
| | | Log(pre-op PSA) | 1 unit increase | 1.89 | 1.31 | 2.75 | 0.0008 | |
| | 6 | | | | | | | 0.86 |
| | | MUC1 | Strong vs. Negative | 1.08 | 0.38 | 3.07 | N/A | |
| | | | Moderate vs. Negative | 0.70 | 0.22 | 2.29 | N/A | |
| | | | Weak vs. Negative | 0.73 | 0.28 | 1.88 | N/A | 0.001 |
| | | Gleason | 3+4 vs. 6 | 2.68 | 1.17 | 6.13 | 0.02 | |
| | | | 4+3 vs. 6 | 3.66 | 1.38 | 9.73 | 0.01 | |
| | | | 8-10 vs. 6 | 6.36 | 2.51 | 16.13 | <.0001 | |
| | | Log(pre-op PSA) | 1 unit increase | 1.91 | 1.31 | 2.77 | 0.0007 | |
| | | | | | | | | |
| RFS | 7 | MUC1 | Strong vs. Others | 1.36 | 0.95 | 1.96 | 0.10 | 0.0002 |
| | | Margin | Positive vs. Negative | 1.64 | 1.33 | 2.04 | <.0001 | |
| | | SVI | Positive vs. Negative | 2.03 | 1.47 | 2.82 | <.0001 | |
| | | ECE | Positive vs. Negative | 1.30 | 1.04 | 1.62 | 0.02 | |
| | | Gleason | 3+4 vs. 6 | 1.20 | 0.94 | 1.53 | 0.14 | 0.0002 |
| | | | 4+3 vs. 6 | 1.92 | 1.43 | 2.58 | <.0001 | |
| | | | 8-10 vs. 6 | 1.49 | 1.06 | 2.07 | 0.02 | |
| | | Log(pre-op PSA) | 1 unit increase | 1.42 | 1.21 | 1.67 | <.0001 | |

3. Lower stromal index number and higher stroma index percent were significantly associated with worse TTBCR after adjusting for margin, SVI, ECE, and pre-op PSA. (Manuscript in preparation)

| | | | | | | |
|---|--------------------|--------------|---------|---------|---------|--|
| <p>Table 3. Summary of <u>multivariate</u> Cox proportional hazard model for time-to-biochemical recurrence (<u>TTBCR</u>) for <u>stromal</u> biomarkers. Event is defined as post-op PSA above 0.2. Hazard ratio higher than 1 means worse prognosis. <u>Conclusions</u>:</p> <ol style="list-style-type: none"> 1. Lower stroma index number and higher stroma index percent were significantly associated with worse TTBCR after adjusting for margin, SVI, ECE, and pre-op PSA. 2. Positive margin, SVI, ECE, and higher pre-op PSA were significantly associated with worse TTBCR. | | | | | | |
| Factor | Comparison | Hazard Ratio | 95% LCL | 95% UCL | P-value | |
| Margin | pos vs. neg | 1.49 | 1.18 | 1.89 | 0.0009 | |
| SVI | pos vs. neg | 1.98 | 1.39 | 2.83 | 0.0002 | |
| ECE | pos vs. neg | 1.52 | 1.20 | 1.93 | 0.001 | |
| stroma Index Num | <468.9 vs. >=468.9 | 1.53 | 1.18 | 1.99 | 0.001 | |
| stroma Index Pct | >=0.26 vs. <0.26 | 1.43 | 1.14 | 1.79 | 0.002 | |
| log(pre-op PSA) | 1 unit increase | 1.57 | 1.32 | 1.87 | <.0001 | |

4. Negative or weak AZGP1 IHC staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, ECE, and Gleason score. Negative or weak AZGP1 CISH staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, and Gleason score. (Manuscript submitted)

| Table 4. Multivariate Cox proportional hazard model for recurrence-free survival (RFS) for AZGP1. RFS event is defined as any recurrence, metastasis, or prostate cancer death. Hazard ratio higher than 1 means worse prognosis. Conclusions: | | | | | | |
|---|-------------------|-----------------------------------|--------------|---------|---------|---------|
| 1. Negative or weak AZGP1 IHC staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, ECE, and Gleason score. | | | | | | |
| 2. Negative or weak AZGP1 CISH staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, and Gleason score. | | | | | | |
| Model | Factor | Comparison | Hazard Ratio | 95% LCL | 95% UCL | P-value |
| 1 (Total #Pts = 835, #Events = 382) | AZGP1 IHC | Negative/Weak vs. Moderate/Strong | 1.39 | 1.13 | 1.71 | 0.002 |
| | Log(PSA) | 1 unit increase | 1.43 | 1.21 | 1.68 | <.0001 |
| | margin | Pos vs. Neg | 1.62 | 1.31 | 2.02 | <.0001 |
| | SVI | Pos vs. Neg | 2.20 | 1.58 | 3.06 | <.0001 |
| | ECE | Pos vs. Neg | 1.26 | 1.01 | 1.58 | 0.04 |
| | Gleason | 3+4 vs. <=6 | 1.19 | 0.93 | 1.52 | 0.16 |
| | | 4+3 vs. <=6 | 1.99 | 1.47 | 2.69 | <.0001 |
| | | 8-10 vs. <=6 | 1.43 | 1.02 | 1.99 | 0.04 |
| 2 (Total #Pts = 811, #Events = 377) | AZGP1 CISH | Negative/Weak vs. Moderate/Strong | 1.28 | 1.04 | 1.58 | 0.02 |
| | Log(PSA) | 1 unit increase | 1.46 | 1.24 | 1.73 | <.0001 |
| | margin | Pos vs. Neg | 1.71 | 1.39 | 2.12 | <.0001 |
| | SVI | Pos vs. Neg | 2.26 | 1.62 | 3.15 | <.0001 |
| | Gleason | 3+4 vs. <=6 | 1.22 | 0.96 | 1.57 | 0.11 |
| | | 4+3 vs. <=6 | 2.12 | 1.57 | 2.86 | <.0001 |
| | | 8-10 vs. <=6 | 1.60 | 1.15 | 2.23 | 0.006 |

5. Ki67 status is significantly associated with OS and DSS (Manuscript submitted)

| Table 5. Summary of multivariate Cox proportional hazard model for recurrence-free survival (<u>RFS</u>), overall survival (<u>OS</u>), and disease-specific survival (<u>DSS</u>) by <u>Ki-67</u> status. | | | | | | |
|---|-------------------------------|-------------------|---------------------|----------------|----------------|------------------------|
| Model | Factor | Comparison | Hazard Ratio | 95% LCL | 95% UCL | Overall p-value |
| RFS Model 1 (N = 634, #Events = 281) | Ki67% weighted average | 1% increase | 1.07 | 1.03 | 1.11 | 0.0008 |
| | Margin | Pos vs. Neg | 1.41 | 1.09 | 1.84 | 0.01 |
| | Seminal vesicle invasion | Yes vs. No | 1.85 | 1.23 | 2.80 | 0.003 |
| | Gleason score | 3+4 vs. 6 | 1.22 | 0.90 | 1.66 | 0.005 |
| | | 4+3 vs. 6 | 1.88 | 1.33 | 2.66 | |
| | | 8-10 vs. 6 | 1.42 | 0.96 | 2.09 | |
| | Pathologic stage | pT3/pT4 vs. pT2 | 1.43 | 1.07 | 1.92 | 0.02 |
| | Log(PSA) | 1 unit increase | 1.62 | 1.35 | 1.96 | <.0001 |
| RFS Model 2 (N = 634, #Events = 281) | Ki67% maximum | 1% increase | 1.04 | 1.01 | 1.07 | 0.007 |
| | Margin | Pos vs. Neg | 1.40 | 1.08 | 1.83 | 0.01 |
| | Seminal vesicle invasion | Yes vs. No | 1.85 | 1.22 | 2.79 | 0.004 |
| | Gleason score | 3+4 vs. 6 | 1.24 | 0.91 | 1.67 | 0.003 |
| | | 4+3 vs. 6 | 1.91 | 1.35 | 2.70 | |
| | | 8-10 vs. 6 | 1.46 | 0.99 | 2.15 | |
| | Pathologic stage | pT3/pT4 vs. pT2 | 1.44 | 1.07 | 1.93 | 0.02 |
| | Log(PSA) | 1 unit increase | 1.61 | 1.34 | 1.95 | <.0001 |
| OS (N = 984, #Events = 57) | Ki67% positive | 1% increase | 1.09 | 1.01 | 1.16 | 0.02 |
| | Gleason Score | 3+4 vs. ≤6 | 0.87 | 0.44 | 1.72 | 0.68 |
| | | 4+3 vs. ≤6 | 1.14 | 0.46 | 2.84 | 0.78 |
| | | 8-10 vs. ≤6 | 3.28 | 1.65 | 6.51 | 0.0007 |
| DSS (N = 874, #Events = 44) | Ki67% positive | 1% increase | 1.10 | 1.02 | 1.18 | 0.02 |
| | Log(PSA) | 1 unit increase | 1.98 | 1.35 | 2.89 | 0.005 |
| | Gleason Score | 3+4 vs. ≤6 | 2.27 | 0.93 | 5.52 | 0.07 |
| | | 4+3 vs. ≤6 | 2.75 | 0.97 | 7.81 | 0.06 |
| | | 8-10 vs. ≤6 | 5.13 | 1.92 | 13.75 | 0.001 |

6. P27 is not significantly associated with RFS after adjusting for clinical predictors
(Manuscript in preparation)

Table 6. Summary of multivariate Cox proportional hazard model results for RFS by p27 status.
RFS event is defined as any recurrence, metastasis, or prostate cancer death post-surgery.
Hazard ratio higher than 1 means worse prognosis. The total sample size for both models was 699, and the number of RFS events observed was 319. Conclusion:

1. p27 was not significantly associated with RFS after adjusting for margin, SVI, Gleason, and pre-op PSA.

| Factor | Comparison | Hazard Ratio | 95% LCL | 95% UCL | P-value |
|-----------------|-----------------|--------------|---------|---------|---------|
| p27HscoreCyto | 1 unit increase | 0.999 | 0.997 | 1.000 | 0.16 |
| Margin | pos vs. neg | 1.61 | 1.28 | 2.03 | <.0001 |
| SVI | yes vs. no | 2.50 | 1.74 | 3.59 | <.0001 |
| Gleason | 3+4 vs. 6 | 1.44 | 1.09 | 1.90 | 0.01 |
| | 4+3 vs. 6 | 2.17 | 1.56 | 3.01 | <.0001 |
| | 8-10 vs. 6 | 1.73 | 1.21 | 2.49 | 0.003 |
| Log(pre-op PSA) | 1 unit increase | 1.57 | 1.32 | 1.86 | <.0001 |
| | | | | | |
| p27HscoreCyto | 1 unit increase | 0.999 | 0.997 | 1.000 | 0.11 |
| Margin | pos vs. neg | 1.62 | 1.29 | 2.03 | <.0001 |
| SVI | yes vs. no | 2.50 | 1.74 | 3.60 | <.0001 |
| Gleason | 3+4 vs. 6 | 1.43 | 1.08 | 1.89 | 0.01 |
| | 4+3 vs. 6 | 2.15 | 1.55 | 2.99 | <.0001 |
| | 8-10 vs. 6 | 1.73 | 1.21 | 2.48 | 0.003 |
| Log(pre-op PSA) | 1 unit increase | 1.56 | 1.31 | 1.86 | <.0001 |

Ongoing studies: See summary on page 3-4. Preliminary analyses completed for ARG2 and CD38, SULT2B, N-glycan. Continuing statistical support for manuscripts reporting MUC1, stromal index, histology pattern, and p27, and second manuscript on PTEN. Expecting statistical analyses once the pathologist scoring completed for CD10, p63, Masson's trichrome.

Specific Aim 2) To evaluate candidate markers that correlate with Gleason grade on prostate cancer tissue microarrays with associated clinical data.

Thus far, we have focused on building the analysis pipeline and in staining high priority biomarkers of prognosis. In all of the biomarkers we have tested thus far, we have interrogated each for its correlation with Gleason score. In general, most of them are correlated, although not completely. While these do not address the intent of this Aim, we are not disappointed since it does appear that *these biomarkers are supplying prognostic information that is independent of Gleason score*. The intent of Aim 2, on the other hand, was to investigate biomarkers that correlate with Gleason grade. Several markers are in our queue and are listed in the original proposal. For some, we are still looking for high quality affinity reagents that provide interpretable staining with limited background. Leading candidates are AGR2, a marker expressed at high levels in Gleason pattern 3 cancers and Monoamine oxidase A, expressed at high levels in Gleason pattern 4 disease. As we get through our candidate prognostic markers (listed above and in the queue) we will refocus on biomarkers that predict Gleason grade. This could be useful in characterizing biopsy samples to predict upgrading.

However, this clinical question might become less relevant in the future since several tools have been developed that already predict up-grading. For example the OncotypeDx assay has been calibrated and already validated precisely for this purpose. In addition, multiparametric MRI shows good correlation with grade in that only the high-grade lesions are visible, while the low grade lesions are not. As the clinical practice evolves, we will decide whether we wish to continue to pursue development of IHC biomarkers that predict Gleason score

For all biomarkers, whether for Gleason score or prognosis, the statistical analysis strategy has been outlined in our proposal and will be used as soon as reads are available from the pathologists, both in their correlations with Gleason score and in their complementary property with Gleason score.

Key Research Accomplishments

- Provided statistical expertise in biomarker review and approval by the investigative team to ensure quality of the reagents and sufficient level of evidence for investigation of a particular biomarker on our valuable resource.
- Data receiving, reconcile data questions, and archiving at MDACC.
- Received final clinical data that will be used for analysis of biomarker performance to the MD Anderson DMCC.
- Established and tested the data analysis pipeline for anticipated additional biomarker data.
- Evaluated TACOMA imaging analysis algorithm using Survivin, CD117, and ERG data and concluded that it is inadequate for automated imaging analysis as it stands along.
- Completion of analysis of PTEN FISH and a manuscript published.
- Completion of analysis of Ki67 PI and submission of a manuscript.
- Completion of analysis of ERG IHC and PTEN IHC and presentation at international meetings and results published.
- Completion of analysis of SPINK and results published.
- Ongoing analysis of AZGP1 with a manuscript submitted.
- Completion of analysis of a modified Gleason grading system with Jesse McKenney, manuscript to be submitted.
- Ongoing analysis of Muc1, p63, CD10 and CD38. We expect all of these, regardless of outcome (prognostic or not) will be submitted as separate publications.
- Significant preliminary data from this collaboration that will position us well for the next phase of funding.

Reportable Outcomes

1) Publications referencing this grant:

James D. Brooks: Translational genomics: The challenge of developing cancer diagnostic biomarkers. *Genome Research* **22**: 183-187, 2012.

Sarah Hawley, Ladan Fazli, Jesse K. McKenney, Jeff Simko, Dean Troyer, Marlo Nicolas, Lisa F. Newcomb, Janet E. Cowan, Luis Crouch, Michelle Ferrari, Javier Hernandez, Antonio Hurtado-Coll, Kyle Kuchinsky, Janet Liew, Rosario Mendez-Meza, Elizabeth Smith, Imelda Tenggarra, Xiaotun Zhang, Peter R. Carroll, June M. Chan, Martin Gleave, Raymond Lance, Daniel W. Lin, Peter S. Nelson, Ian M. Thompson, Ziding Feng, Lawrence D. True and James D. Brooks: Design and construction of a resource for the validation of candidate prognostic biomarkers: the Canary Prostate Cancer Tissue Microarray as a model. *Advances in Anatomic Pathology* **20**: 39-44, 2013.

J James D. Brooks: Managing localized prostate cancer in the era of prostate specific antigen testing. *Cancer* **119**: 3906-3909, 2013.

Zuxiong Chen, Zulfiqar G. Gulzar, Catherine A. St. Hill, Bruce Walcheck, James D. Brooks: Increased expression of *GCNT1* is associated with altered O-glycosylation of PSA, PAP and MUC1 in human prostate cancers. *Prostate* **74**: 1059-1067, 2014.

Troyer D, Jamaspishvili T, Wei W, Feng Z, Good J, Hawley S, Fazli L, McKenney J, Simko J, Hurtado-Coll A, Carroll P, Gleave M, Lance R, Lin D, Nelson P, Thompson I, True L, Brooks J, Squire J. A multicenter study shows PTEN deletion is strongly associated with seminal vesicle involvement and extracapsular extension in localized prostate cancer. *The Prostate*. 75(11): 1206-1215, 2015.

James D. Brooks, Wei Wei, Sarah Hawley, Heidi Auman, Lisa Newcomb, Hilary Boyer, Ladan Fazli, Jeff Simko, Antonio Hurtado-Coll, Dean A. Troyer, Peter R. Carroll, Martin Gleave, Raymond Lance, Daniel W. Lin, Peter S. Nelson, Ian M. Thompson, Lawrence D. True, Ziding Feng and Jesse K. McKenney. Evaluation of ERG and SPINK1 by immunohistochemical staining and clinicopathological outcomes in a multi-institutional radical prostatectomy cohort of 1067 patients. *PLOS ONE* 10(7): e0132343. Doi:10.1371/journal.pone.0132343.

Conclusion

We have undertaken a challenging task of creating a multi-institutional TMA resource with rigorous case/cohort design. To our knowledge, such a resource has not been previously created and offers the advantage of reducing institutional biases as well as spectrum biases. In the uniform design and through image acquisition and archiving technologies, we have created a resource that can be easily used by the greater prostate cancer research community. In many ways, this resource represents a gold standard by for evaluation of prognostic biomarkers. We have completed all phases of

pipeline construction and continue to refine our work-flow to improve functionality as we work with the resource. We now have tested several biomarkers and confirmed that they are prognostic. We will complete analysis of the biomarkers in the context of the clinical data over the next year and plan several publications. In addition, we will continue to carry out analysis of new biomarkers and solicit applications for biomarkers inside and outside our research group. This research directly addresses the PCRP overarching challenge to *distinguish lethal from indolent disease*.